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PRIMER NOTE

Polymorphic microsatellite loci from the western corn rootworm (Insecta: Coleoptera: Chrysomelidae) and cross-amplification with other *Diabrotica* spp

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Abstract

Corn rootworms (*Diabrotica* spp.) make up the major insect pest complex of corn in the US and Europe, and there is a need for molecular markers for genetics studies. We used an enrichment strategy to develop microsatellite markers from the western corn rootworm (*Diabrotica virgifera virgifera*). Of 54 loci isolated, 25 were polymorphic, and of these, 17 were surveyed for variability in 59 wild individuals. In addition, the potential for cross-amplification of these microsatellites was surveyed for Mexican, northern, and southern corn rootworms. Nine microsatellite loci showed Mendelian inheritance and are likely to be useful in population genetics studies.

Keywords: *Diabrotica virgifera*, microsatellites, molecular markers, population genetics, western corn rootworm

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The western (*Diabrotica virgifera virgifera* LeConte), Mexican (*Diabrotica virgifera zeae* Krysan & Smith), northern (*Diabrotica barberi* Smith and Lawrence), and southern (*Diabrotica undecimpunctata howardi* Barber) corn rootworms represent the main pest complex of corn in the United States (Levine & Oloumi-Sadeghi 1991), causing losses of over $1 billion annually. The evolution of resistance to insecticides and crop rotation, the recent introduction and spread of the western corn rootworm in Europe, and the large-scale commercial deployment of rootworm-active transgenic corn in North America have generated growing interest in *Diabrotica* genetics research and the need for molecular markers. We report the development of microsatellites for the western corn rootworm and the results of cross-amplification of these markers in the other three rootworms.

Microsatellite loci were isolated by the biotin-enrichment methods of Kijas *et al*. (1994) and Ronald *et al*. (2000) with slight modifications as described in detail by Kim & Sappington (2004). Briefly, pooled genomic DNA from 50 rootworm adults was digested with *Nde*II, and DNA fragments 250–600 bp long were purified. The biotinylated capture probe was annealed to the DNA fragments linkered with an *Nde*II compatible end (Ronald *et al*. 2000), incubated with magnetic beads, and washed with 1X SSC at an optimized temperature [70 °C for $(CA)_{15}$, 63 °C for $(CT)_{15}$, 68 °C for $(AGC)_7$, 58 °C for $(GAG)_7$, 60 °C for $(GATA)_6$]. The DNA was reamplified by PCR, the desired size fragments purified and ligated into pGEM T-vector (Promega), and the plasmid DNA isolated.

A total of 324 clones (268 dinucleotide repeat clones and 56 trinucleotide repeat clones) were sequenced using a Beckman-Coulter CEQ 8000 Genetic Analysis System, and 54 clones were finally chosen to design microsatellite primer sets. The 5′-end of forward PCR (polymerase chain reaction) primers were labelled with Beckman-Coulter phosphoramidite flourophores enabling detection by the automated sequencer. An exception was the *DVV-D8* marker, where the reverse primer was labelled.

Preliminary screening of the 54 microsatellites revealed that 25 were polymorphic. Subsets from among 17 of these were tested for compatibility in PCR multiplexing (Table 1). Primer sets for microsatellites with similar size ranges were labelled with different coloured dyes. Reaction solutions consisted of 15 ng genomic DNA, with 1.25 μL 10X thermal polymerase buffer [10 mM Tris-HCl (pH 9.0 at 25 °C), 50 mM KCl and 0.1% Triton X-100] (Promega), 1.5 mM $MgCl_2$, 200 μM each dNTP, 0.16 μM each primer,

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Table 1 Characteristics of polymorphic microsatellites from *Diabrotica virgifera virgifera* in a survey of 59 wild individuals. H_O, observed heterozygosity. H_E, expected heterozygosity

Locus	Primer sequences (5′–3′)	Repeat motif	No. of alleles	Size range	H_O	H_E	P*	GenBank accession no.
DVV-D1	F: AGACGAAGTGAGAGCCGATG R: CCATCCTCAATCTGTGGCTAA	$(GT)_{11}$	9	100–132	0.678	0.732	0.8795	AY738544
DVV-D2	F: CACGCAGCACTTAATTGGTTT R: CTATGCCTCCCAATTCGTGT	$(CA)_{16}$	9	182–208	0.678	0.780	0.1619	AY738532
DVV-D3	F: CCTTGTCTGCTTTGGCAGTA R: TCCATCGCCCTTTGAGATAC	$(GT)_{11}$	14	207–259	0.759	0.880	0.0047	AY738533
DVV-D4	F: TGTGTGCAGTGTCCCGTTAT R: GTGGCCAGTATTCACGACCT	$(GT)_{12}$	7	221–239	0.712	0.686	0.5340	AY738534
DVV-D5	F: TCCGGAAATCTCATGTCCTC R: TTCATCGCGTGTCCATTTTA	$(GT)_8AT(GA)_2$	3	173–177	0.237	0.229	0.3736	AY738535
DVV-D6	F: GGAGAATAAATTCTGTGGCAAAA R: TCATTTGGCAGCTTTCTAGC	$(CA)_{10}$	5	183–197	0.288	0.463	0.0000	AY738536
DVV-D7	F: CTGCGTGTGAACTTGTTGCT R: ATCAGACCGTCTTGTTGTCG	$(GA)_6AA(GA)_{29}$	11	133–169	0.535	0.790	0.0000	AY738537
DVV-D8	F: AAGGCAGGTAGTAATGTTGGTGA R: TCATCACTAATGGGGAAACGA	$(CT)_{22}$	20	211–249	0.881	0.881	0.2301	AY738538
DVV-D9	F: GGCATAAGCGCTGCATAAGT R: TCTCTGGGCATTGTTTAGGG	$(CA)_9$	5	139–153	0.509	0.576	0.0459	AY738539
DVV-D10	F: TGACGTTTAGACCGTCAGAAAA R: GATCGCTACTGTTCGCTCTT	$(GT)_{17}$	11	135–163	0.780	0.824	0.8374	AY738540
DVV-D11	F: GGCTGGGGTTGATACGAG R: CGTAACCTTCATATCCGAGCA	$(GT)_{16}GC(GT)_5$	12	171–211	0.814	0.839	0.1618	AY738541
DVV-D12	F: GGAAACTCATTCTGCGAAGC R: TGTCGCCATTTACGTTTCTATT	$(GA)_{31}$	11	191–235	0.593	0.840	0.0000	AY738542
DVV-D13	F: TCTGTGGCAAATAAGACATGC R: CTTGGCAGCCATCTGAAATC	$(CA)_{34}$	13	89–147	0.661	0.843	0.0006	AY738543
DVV-T1	F: GATCAGAACTTTGGAATCTCTCC R: TCCTCTTAAGGGTTTCGTGTG	$(AGC)_{11}$	4	203–218	0.593	0.723	0.0046	AY738545
DVV-T2	F: ATCGGTTTTGGCTGGATATG R: GTTCAACAACTCGCAAACCA	$(GCT)_{13}$	3	212–224	0.509	0.508	0.8279	AY738546
DVV-T3	F: GTTGCTGTTGTTGGGAAGGT R: TTGAGAGGAAGCAACAACCTC	$(GCT)_7$	2	223–241	0.362	0.378	0.7347	AY738547
DVV-T4	F: GGAACAGGACAAGACCCAAA R: GCATCCTTTTCCTTGACTGC	$(TGA)_{13}$	5	203–218	0.357	0.557	0.0000	AY738548

*Hardy–Weinberg exact test, GENEPOP version 3.1b (Raymond & Rousset 1995).

and 0.5 unit *Taq* polymerase (Promega) in a total volume of 12.5 μL. The PCR conditions consisted of denaturation for 3 min at 94 °C; followed by 30 cycles of 1 min at 94 °C; 1 min at 57 °C; and 1 min at 72 °C, and a final extension at 72 °C for 10 min. Multiplexing was successful for the subsets *DVV-D1* + *DVV-D9* + *DVV-D13*, *DVV-D2* + *DVV-D12*, *DVV-D4* + *DVV-D5*, *DVV-D7* + *DVV-D8* + *DVV-T1*, *DVV-D11* + *DVV-T2*, *DVV-D3* + *DVV-D6* + *DVV-D10*, *DVV-T3* + *DVV-T4*.

Variability of the 17 microsatellites was surveyed in 59 wild western corn rootworm individuals collected in central Iowa in 2003 (Table 1). The number of alleles per locus ranged from two in *DVV-T3* to 20 in *DVV-D8*, and expected heterozygosity values ranged from 0.229 in *DVV-D5* to 0.881 in *DVV-D8*. Eight of the 17 loci showed significant deviations from Hardy–Weinberg proportions ($P < 0.05$) (Table 1). After correction for multiple tests [adjusted significance (5%) threshold = 0.00294], five markers still exhibited deviation from Hardy–Weinberg equilibrium (HWE).

In a preliminary analysis of four families (25 offspring per family), seven markers, *DVV-D1*, *DVV-D3*, *DVV-D6*, *DVV-D7*, *DVV-D12*, *DVV-D13*, and *DVV-T1* did not display a Mendelian mode of inheritance in that at least one offspring did not exhibit one of the parents' alleles. *DVV-T4* primers did not amplify DNA from two wild WCR individuals despite other markers being amplified well. All these markers, except *DVV-D1*, deviate significantly from Hardy–Weinberg proportions in the direction of heterozygote deficiency (Table 1). Thus, the primers for these markers are not likely to be useable in population studies

Table 2 Cross-taxon amplification using western corn rootworm (*Diabrotica virgifera virgifera*) microsatellites

Locus	*Diabrotica virgifera zeae* PCR method*	*Diabrotica virgifera zeae* Size range (no. alleles)	*Diabrotica barberi* PCR method	*Diabrotica barberi* Size range (no. alleles)	*Diabrotica undecimpunctata howardi* PCR method	*Diabrotica undecimpunctata howardi* Size range (no. alleles)
DVV-D1	57 °C	102–108 (2)†	—	—	—	—
DVV-D2	57 °C	180–184 (2)	—	—	—	—
DVV-D3	57 °C	173–241 (7)	—	—	—	—
DVV-D4	57 °C	221–237 (6)	TD	N.S.‡	—	—
DVV-D5	57 °C	169–175 (3)	TD	N.S.	TD	N.S.
DVV-D6	57 °C	183–193 (2)	—	—	—	—
DVV-D7	57 °C	107–161 (5)	—	—	—	—
DVV-D8	57 °C	229–237 (5)	57 °C	167–185 (4)	—	—
DVV-D9	57 °C	139–151 (4)	—	—	TD	N.S.
DVV-D10	57 °C	125–153 (5)	TD	N.S.	—	—
DVV-D11	57 °C	169–229 (7)	TD	140 (1)	—	—
DVV-D12	57 °C	193–231 (5)	57 °C, TD	N.S.	57 °C, TD	N.S.
DVV-D13	57 °C	91–141 (3)	—	—	—	—
DVV-T1	57 °C	206–209 (2)	TD	220–226 (2)	TD	146–158 (3)
DVV-T2	57 °C	206–224 (4)	57 °C	197 (1)	57 °C	197–203 (2)
DVV-T3	57 °C	238–241 (2)	57 °C	205–244 (5)	57 °C, TD	N.S.
DVV-T4	57 °C	203–209 (2)	TD	185–221 (2)	TD	N.S.

*PCR was first attempted with an annealing temperature of 57 °C. If unsuccessful, a touchdown (TD) protocol was attempted (see text for detail); †Based on survey of five individuals from each taxon; ‡N.S. Nonspecific bands, where exact sizing of bands is difficult.

and will need to be redesigned. Although the occurrence of null alleles cannot be excluded for the other markers, 9 of the microsatellites appear to be inherited in a Mendelian fashion and are promising for population genetics studies of the western corn rootworm.

We tested the 17 microsatellites developed in this study for cross-amplification of DNA from Mexican corn rootworm (*D. virgifera zeae* Krysan & Smith), northern corn rootworm (*D. barberi* Smith and Lawrence), and southern corn rootworm (*D. undecimpunctata howardi* Barber). Each primer set was analysed against a panel of five individuals from each taxon. PCR reactions were carried out using a single marker per reaction tube. Initially, PCR was conducted at an annealing temperature of 57 °C. If a clear amplification product was not observed on a 1.7% agarose gel in 0.5 X TBE buffer, PCR was tried again using the touch down method, conducted with a one-degree reduction at each cycle from 60 °C to 53 °C, followed by 28 cycles at an annealing temperature of 53 °C.

Of the 17 microsatellites examined for cross-amplification, all amplified DNA of the Mexican corn rootworm, a subspecies of the western corn rootworm, and six amplified DNA of the northern corn rootworm (Table 2). In contrast, only two markers (*DVV-T1* and *DVV-T2*) amplified DNA of the southern corn rootworm. Primers for many markers produced nonspecific bands in northern and southern corn rootworms. Accordingly, various types of PCR conditions, such as different concentrations of $MgCl_2$ and/or less stringent annealing temperatures, may improve the success rate of cross-amplification of DNA from these two species.

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References

Kijas JM, Fowler JC, Garbett CA, Thomas MR (1994) Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. *Biotechniques*, **16**, 657–662.

Kim KS, Sappington TW (2004) Isolation and characterization of polymorphic microsatellite loci in the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). *Molecular Ecology Notes*, **4**, 701–703.

Levine E, Oloumi-Sadeghi H (1991) Management of diabroticite rootworms in corn. *Annual Review of Entomology*, **36**, 229–255.

Raymond M, Rousset F (1995) GENEPOP: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ronald JS, David VA, Franklin WL, O'Brien SJ, Johnson WE (2000) Development of microsatellite markers in the guanaco, *Lama guanicoe*: utility for South American camelids. *Molecular Ecology*, **9**, 1922–1924.